DISCUSSION PAPER

Alleles and algorithms: The role of genetic analyses and remote sensing technology in an ant eradication program

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Abstract

Eradication programs for invasive ants are often hampered by a lack of effective tools to detect, contain and kill the pests. Among the range of tools employed in the course of a 20-year eradication program for red imported fire ant, *Solenopsis invicta*, in Australia, two of the most crucial for success are genetic analysis at both individual colony and population scales, and remote sensing for the detection of *S. invicta* mounds over large areas. Several genetic analyses are used by the program as an everyday operational tool to guide the eradication effort; for example, genotyping of the social form determines where and how far we need to search and treat, whereas nest relatedness derived from microsatellites aids in deciding when and where to target investigations into human-assisted movement of the pest. Microsatellite genotyping can determine the origin of new invasions into the country and has been used to verify the eradication of six distinct incursions of *S. invicta* in Australia, as well as demonstrating the pressure being exerted on the remaining Queensland population by the current eradication activities. Remote sensing played a key role in delimiting the extent of the *S. invicta* infestation in southeast Queensland in 2015, and in the future will assist in both delimitation and in verifying eradication of this ant in treatment areas as part of the proof of freedom process. Unquestionably, without these tools, the battle to eradicate *S. invicta* from Australia would be severely constrained, if not lost. These technologies may be applicable in management or eradication programs for *S. invicta* worldwide, and potentially for other invasive ant species.

Keywords

Invasive ants, red imported fire ant, Solenopsis invicta

Introduction

The red imported fire ant, *Solenopsis invicta* Buren is a notorious invasive ant species which has become established in several countries around the world, causing economic and ecological damage and affecting health and lifestyle (Wetterer 2013). In Australia, a national program (the program) for the eradication of *S. invicta*, has been in operation since 2001 when the ant was first discovered at two locations in Brisbane, Queensland (Vanderwoude et al. 2003). Responses to these and subsequent incursions in other parts of Australia are part of the overarching national program. The methodology employed in this program and some of its successes have been outlined by Wylie et al. (2016). Six of the nine known incursions of *S. invicta* into Australia have been eradicated (five in Queensland and one in New South Wales), including the world's largest ant eradication which was one of the original infestations at the Port of Brisbane (8300 hectares) (Hoffmann et al. 2016; Wylie and McNaught 2019). Efforts continue against the remaining southeast Queensland infestation, which now covers an area of over 500 000 hectares. Recently, at the end of 2019, an incursion of *S. invicta* was detected at the Port of Fremantle in Western Australia from China, and another at the Port of Brisbane from the United States in early 2021. Localised eradication responses have commenced for both incursions.

While eradication of *S. invicta* from Australia has not yet been achieved, and aside from the new incursions at the ports of Fremantle and Brisbane, a measure of the efficacy of the program is that the remaining southeast Queensland infestation has been successfully restricted to a relatively small area of the State, with no known spread to other parts of the country from this population. In 2015, the extent of this infestation was delimited by Keith et al. (2019) with a 99.9% level of confidence, and over the life of the program its rate of spread has averaged 4.8 km per year (Wylie et al. 2019). In comparison, the rate of spread of *S. invicta* in Texas over a 10-year period was 48 km per year (Hung and Vinson 1978) and 80 km per year in China (Lu et al. 2008). The slow rate of spread in Australia has been attributed to the program's surveillance and treatment regimes, community support, and strict controls on the movement of products likely to harbour *S. invicta* (Wylie et al. 2019).

These achievements have been substantiated through the use of two of the program's most valuable tools against the pest – genetic analyses to determine origin of new invasions, social form and population genetic structure, and remote sensing for detection of *S. invicta* mounds. The manner in which these tools are employed to inform and guide program strategy and management decisions is described in this paper, along with a case study that demonstrates the benefits of genetic analyses.

Genetic analysis

Social form

Solenopsis invicta colonies contain either a single queen (monogyne) or multiple queens (polygyne), whereas many other of the world's invasive ant species are solely polygynous

(Tsutsui and Suarez 2003). In its native range, *S. invicta* colonies are mostly monogyne, with occasional polygyne colonies that typically contain several closely related queens (Ross et al. 1996). In the United States, it wasn't until the 1970s that polygyne colonies, often containing many unrelated queens, were officially recognised (Ross et al. 1996). Early speculation on polygyny was that it developed in introduced populations of *S. invicta* in response to ecological constraints (e.g., Keller 1995). More recent studies suggest a separate introduction of the polygyne form into North America subsequent to the establishment of monogyne *S. invicta* (Tsutsui and Suarez 2003; Shoemaker et al. 2006).

The social form of a colony/nest is determined in a laboratory by genotyping of the *Gp9* locus from DNA extracted from 10 whole worker ants (pooled) from the same sample and subjected to High Resolution Melt (HRM) Polymerase Chain Reaction (PCR) (Oakey et al. 2011). This discerns the presence of the *Gp9* alleles *B* and *b*. Where all workers are found to be homozygous *BB* it is concluded that the colony is monogynous, whereas the presence of the *b* allele indicates the colony is polygynous. In Brisbane, genotyping revealed that both social forms of the ant were introduced in 2001 at the Port of Brisbane and at Richlands in the west of the city (Wylie et al. 2016).

The reproductive forms display different physical, behavioural, reproductive, and genetic traits. For instance, monogyne colonies spread predominantly by flight while polygyne colonies primarily spread by budding off a new colony overground (King et al. 2009) or by movement of infested materials. As such, determining social form of populations and individual colonies assists in assessing the level of spread risk posed by infestations and facilitates appropriate eradication activities. The program adapts its response depending on the social form; for example, surveillance buffers for a monogyne detection (with strong-flying founding queens) are greater than for a polygyne detection (that predominantly spread only short distances by budding or flight). However, polygyne colonies are of particular concern for several reasons, both in terms of impact to their surroundings and spread risk. Specifically, the higher nest densities in polygynes pose a greater risk to human and animal health, economic impact and the environment. As polygynes have multiple queens – nearly 700 have been recorded in a single mound in the United States (Glancey et al. 1975) – there is a higher likelihood of spread by human assistance (King et al. 2009) in soil or other carriers and by flooding (Biosecurity Queensland, unpublished data) than for monogynes. Additionally, due to the nest density and hierarchical feeding strategy (Tschinkel 2006) they take longer to kill (e.g., an average 4–5 rounds of insect growth regulator bait treatments compared to 3–4 rounds for monogynes (Biosecurity Queensland, unpublished data)).

For the reasons outlined, elimination of polygynes from an *S. invicta* population is an important contributor towards its successful eradication. Monitoring by the program of the distribution and the frequency of polygyne colonies in southeast Queensland shows that there has been a significant reduction in the proportion of polygynes in the population from almost 40% in 2001 to approximately 1% in 2018–2019 (Fig. 1). This reduction has been attributed to targeting of polygyne infestations by the program through chemical treatment and controls on product movement. In comparison, in countries without effective controls or eradication programs for *S. invicta*,
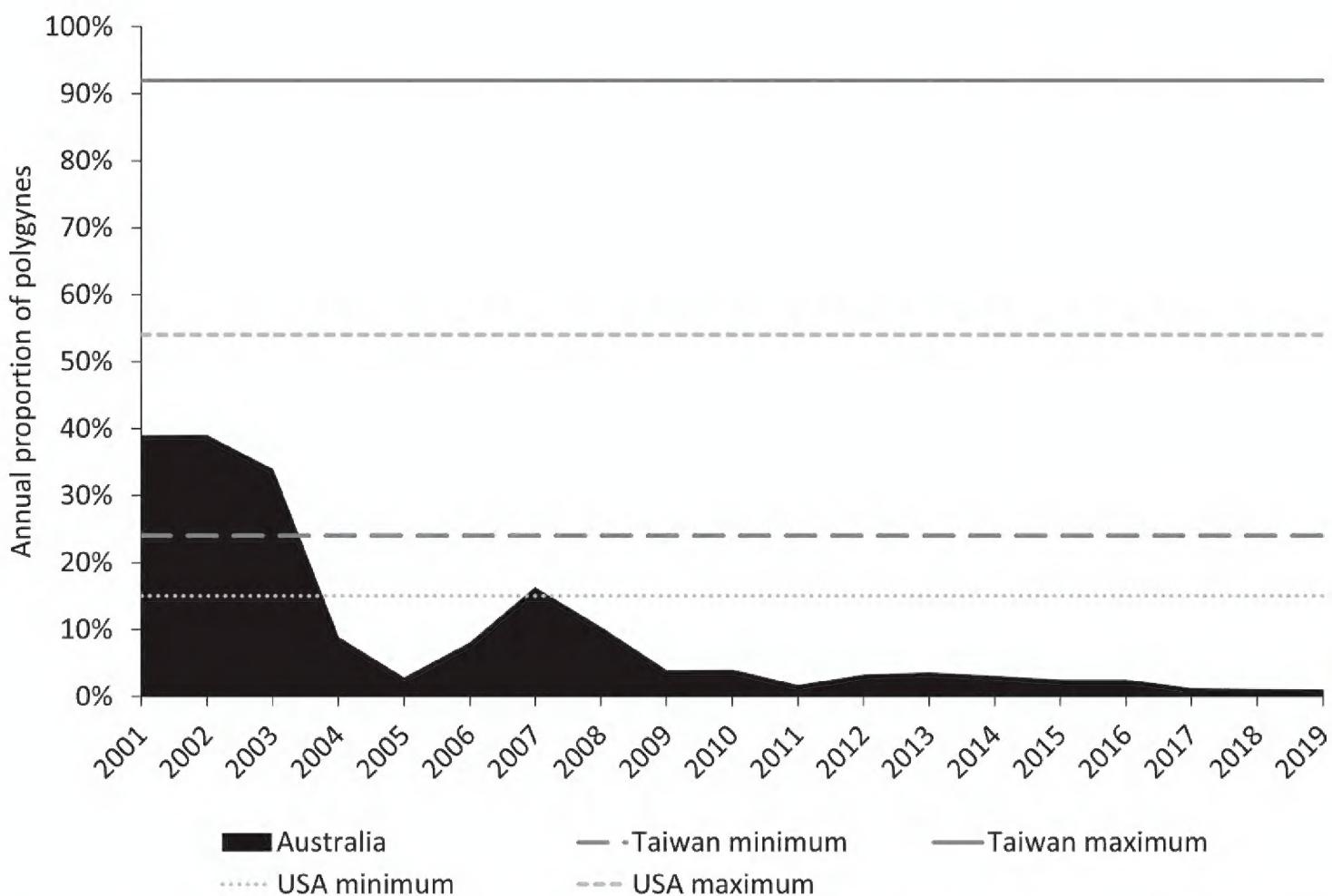
Figure 1. Proportion of sites with the polygyne social form of *S. invicta* in the Brisbane populations. Brisbane data are from the period 2001 to 2019, with the minimum and maximum of this social form in Taiwan and USA populations also portrayed.

the incidence of polygyny is 24–92% in Taiwan (Yang et al. 2008), 15–54% in the United States (Porter et al. 1991; Porter 1992) and in China the majority of colonies are polygyne (Yang et al. 2012).

Occasionally, the determination of a colony as polygyne is unexpected, either because they have not been found in an area before, are found in isolation, or are detected in an area where monogyne colonies predominate. From the perspective of determining the illegal movement of fire ant carriers or tracing the source of the infestation, it is of benefit to determine whether these 'nests of interest' could be a result of occasional, long-distance flight events of polygynes or the more likely movement of material containing queens or alates. In such cases, 10 individual workers are genotyped with HRM PCR. If the workers of a polygyne nest are determined to have a ratio of *Bb:BB:bb* alleles represented by approximately 2:1:1 (or at least to show a mixture), then this is indicative of an established polygyne colony that is either a) likely to have other undiscovered nest mounds in the same area, or b) from a rebuilt nest following assisted movement of one or more queens. Both of these situations require further responsive actions from the program beyond elimination of that nest. However, if all workers are determined to have a *Bb* genotype, it is possible that the nest was established by an occasional surviving *BB* alate (mated winged queen) from a polygyne colony, referred to as a 'heavy queen' because of her higher body weight compared to a *Bb* polygyne alate.

Most heavy queens are executed by the colony prior to flight and therefore seldom survive to undertake a nuptial flight (see Tschinkel 2006). Alternatively, the nest may have been founded by a monogyne queen that mated with a polygyne *b* male. In southeast Queensland, the majority (approximately 80%) of these 'nests of interest' are considered movement of fragments from established nests with the remaining 20% being a nest founded through a heavy queen or monogyne queen mating with a *b* male. As those colonies genotyped are a subset of the total number of polygyne colonies detected, it is concluded that nest founding by heavy queens occurs at only a low frequency. The presence of nests with this *Gp9* genotypic composition (i.e., all workers with a *Bb* genotype) highlights the potential risk of mating events between polygynes and monogynes as a source of genetic variation and the associated risk to the current genetic bottleneck within the southeast Queensland population (discussed further below).

Population structure

Genetic analysis of population structure can provide information on changes in the genetic diversity of populations of the ant. A reduction in genetic variation and the occurrence of inbreeding or population fragmentation may result in reduced fitness and adaptability of the population and indicate program success. A single ant worker from each monogyne colony sampled is genotyped at thirty-seven microsatellite loci. These loci were selected from the 72 used in the global study by Ascunce et al. (2011) as those that show allelic diversity in the Queensland populations. At the time of writing, this database consists of over 36000 colony genotypes. Fragment analyses are performed with Applied Biosystems 3500xL genetic analyser. Microsatellite alleles are determined and proofread using commercial software Genemarker (Soft Genetics via Millennium Science, VIC.) (Ascunce et al. 2011).

Every 1–2 years, the accumulated and annual genotype data are analysed with Bayesian clustering algorithms via the software Structure (Pritchard et al. 2000) to establish the number and spatial distribution of genotypic clusters. A cluster is defined as a collection of genotypes that are more similar to each other than to genotypes outside the cluster. The number of clusters is tested using two methods, (Pritchard et al. 2000 and Evanno et al. 2005). Once the number of clusters is identified, multiple simulations are run with a high number of iterations to assign a probability of each sample belonging to each cluster. Simulations are averaged across runs to a single assignment probability table using CLUMPP software (Jakobsson and Rosenberg 2007).

Long-term analyses of the clusters have shed light on the change in structure of the population over time, and the success of the program. The proportion of the total genetic variance contained in a subpopulation relative to the total genetic variance (F_{ST}, calculated by GenAlEx; Peakall and Smouse 2006) showed that in the first five to six years following discovery of the ant in Queensland (2001–2006), the main Brisbane population consisted of a single genetic cluster ('Main'), the Port of Brisbane population having been eradicated pre-2005–06 (Wylie et al. 2016). In 2006–07 a subcluster emerged in the west of the infestation ('West') and in 2009–10 another sub-
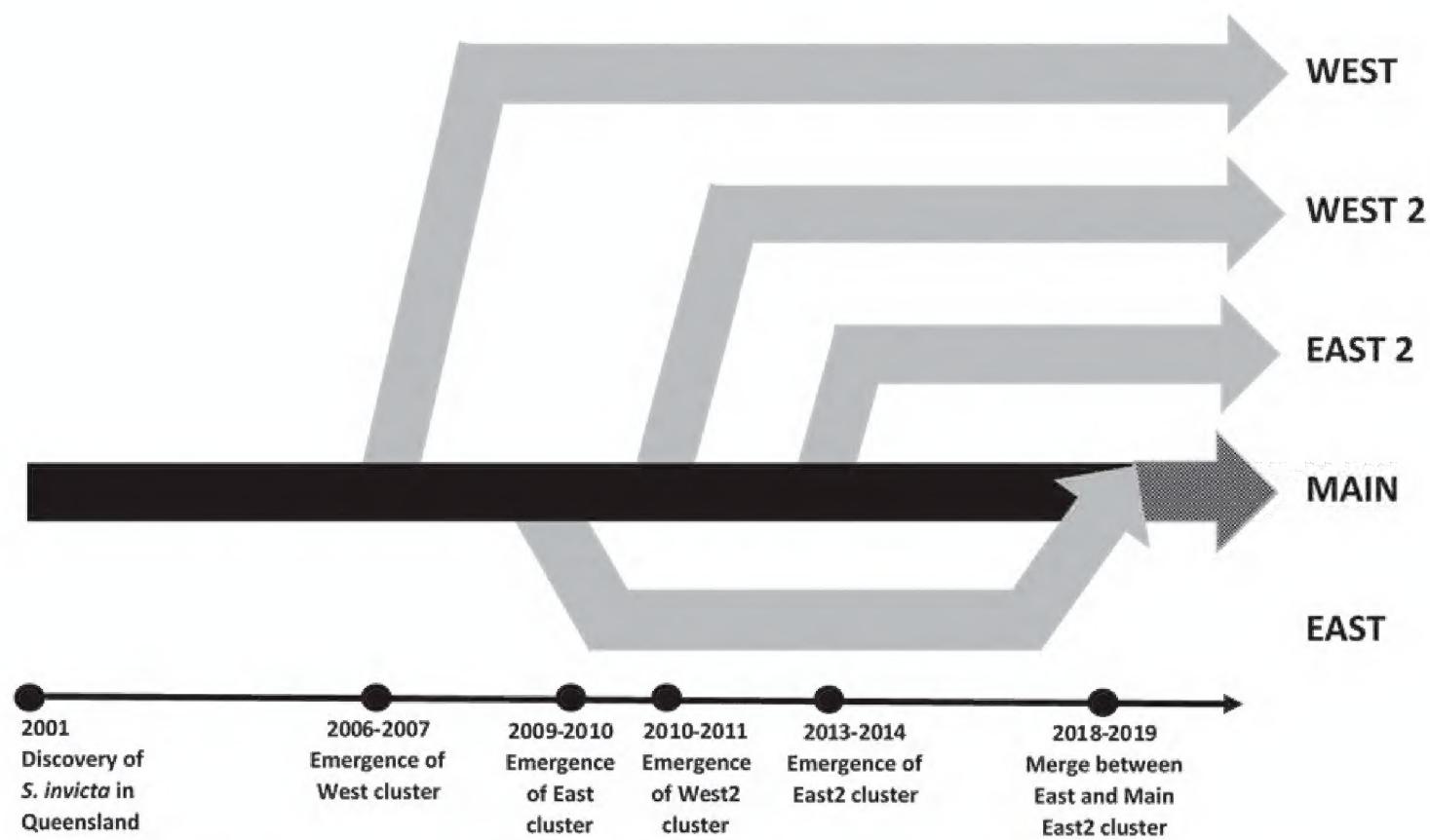
Figure 2. Schematic summarizing the temporal fluctuations of sub-clusters of the Brisbane population of *S. invicta* over the period 2001 to 2019.

cluster emerged in the east ('East'). A second western sub-cluster ('West 2') emerged in 2010–11 from the Main cluster, not from the West sub-cluster. Similarly, a second eastern sub-cluster ('East 2') emerged in 2011–2012 from the Main cluster (Fig. 2).

The formation of these sub-clusters may be indicative of the pressure being exerted on the remaining Brisbane population by the program's eradication efforts. The expectation for a *S. invicta* incursion after 20 years was that there would be no decrease in genetic variation and limited sub-structuring of the population due to genetic mixing via natural mating, migration, and human-assisted transport. The opposite of this is occurring in Queensland where there is lower genetic diversity than is reported in other invaded countries, evidence of inbreeding and population fragmentation. This has not been observed in *S. invicta* populations in countries without effective control or eradication programs (Ascunce et al. 2011). In 2018–2019, the East sub-cluster was no longer distinguishable from the Main which may indicate a weakening of the genetic bottleneck (see below) in that sub-cluster.

Population assignment

When *S. invicta* is detected well outside known areas of infestation, an immediate concern is whether this is spread from an existing population or a new incursion. To address this critical question, there is an extensive global database on the genetics of the ant from which comparisons can be made, again using microsatellite markers (Ascunce et al. 2011). A sample of 10 individuals is genotyped using the microsatellites as above. The alleles are compared to the global data and potential sources are eliminated

if the test sample contains alleles that are not associated with that population. Paetkaus assignment tests in GenAlEx (Peakall and Smouse 2006) are applied to consider the likelihoods of assignment to the different sources.

Using this global database, the assignment tests have enabled the program to pinpoint the origin of all incursions to date. The two 2001 Brisbane incursions, and incursions at the Port of Gladstone in 2013, Brisbane Airport in 2015, and Port of Brisbane in 2021, were from the southern United States. The incursions at Yarwun in 2006, Port Botany in 2014 and Port of Brisbane in 2016 were from Argentina, and the 2019 Fremantle incursion was from China. None of the incursions post-2001 were related to the original Brisbane populations or to each other. Such information allows the program to prove that the subsequent incursions were not as a result of spread from southeast Queensland or that the Port of Gladstone infestation was not the result of eradication failure at the nearby earlier Yarwun incursion. It also shows that the program generally has been effective in containing spread of populations to other parts of Australia.

Relatedness

Determining relationships, particularly parentage, between individual *S. invicta* colonies can provide a range of information that is used by the program for strategic or operational purposes.

Examples of the type of information derived include: (a) providing data on the distances flown by newly mated monogyne *S. invicta* queens for input to models used to predict spread; (b) providing spatial information on the direction and rate of spread of monogynes to guide treatment and surveillance activities; (c) assisting in identifying potential cases of non-compliance with movement restrictions; (d) differentiating between reinfestation of a previously infested area and persistence of a colony following pesticide treatment of that area.

Relatedness estimates are derived from the R-coefficient in haplodiploid (see Bourke and Franks 2019) models. The genotype of the monogyne queen is determined from 10 individual workers and analysed using the software Kingroup (Konovalov et al. 2004), along with manual confirmation through comparison of alleles (see Case Study below).

Genetic bottleneck

When a population undergoes a reduction in its numbers, there is typically a reduction in genetic variation through a loss of rare alleles which can be revealed by microsatellite genotyping of loci that are not under selection (i.e. neutral with respect to selection). When a low number of individuals from a stable population establish in a new area (as with incursions of invasive pests such as *S. invicta*), this new population undergoes an extreme form of bottleneck, referred to as the 'founder effect' (Mayr 1954; Tsutsui and Suarez 2003). A reduction in genetic diversity generally is associated with negative effects that put strain upon the survival of the reduced population. Examples include

reduced ability to adapt or evolve to new situations, reduced capacity to resist parasitoids or disease, and a necessity to breed with kin thus exacerbating these effects (Garza and Williamson 2001; Schmid-Hempel et al. 2007). Therefore, establishing the bottleneck of the *S. invicta* populations and sub-populations on an annual basis provides information relating to the effective pressure on, or recovery of, 'genetic fitness'.

Detection of a bottleneck is made through comparing the expected heterozygosity (see Gregorius 1978) with the observed heterozygosity, and the expectation that populations in genetic bottleneck would show an average heterozygosity excess when compared with the source population or population in genetic equilibrium (Maruyama and Fuerst 1985). On the other hand, a heterozygosity deficit may indicate signs of establishment or recovery. Levels of heterozygosity are determined annually by applying the software Bottleneck (Cornuet and Luikart 1996) to datasets from each of the clusters defined by Structure. Results of analysis in 2019 show that all remaining clusters within the Brisbane population remain in strong bottleneck. All clusters are showing increasing or maintained pressure over time and allele frequencies show evidence of inbreeding, which implies increased stress and reduction in fitness (Garza and Williamson 2001). These analyses also revealed that the Port of Brisbane incursion that was discovered in 2001, pre-dated the Richlands incursion discovered in the same year. Specifically, the early Port of Brisbane data show a recovering population post-establishment, which later demonstrated extreme fitness stress (Biosecurity Queensland, unpublished data) following extensive, repeated treatment that resulted in eradication of this population (Wylie et al. 2016).

Case study: Port of Gladstone 2013

An example of how the program uses genetic analysis to effectively respond to a significant new discovery of *S. invicta* is the 2013 incursion at the Port of Gladstone in central Queensland. Genetic analyses of samples collected at the port facility and nearby industrial sites during the investigative stage of the response showed that all samples were monogyne and the origin was determined as the southern United States. The incursion was unrelated to either of the Brisbane populations or to the 2006 incursion at nearby Yarwun (approximately 4 km away), which originated from Argentina. The knowledge that the colonies were monogyne helped to determine the extent of the surveillance zone. Research in the United States showed that 99% of newly mated queens of monogyne *S. invicta* flew less than 1.6 km unaided by wind (Markin et al. 1971) and an energetics study by Vogt et al. (2000) estimated their flight capability, and ability to successfully found a nest, at <5 km in the absence of wind. Surveillance at the Port of Gladstone was therefore conducted out to a 6 km radius from confirmed infestation to delimit the extent of the incursion. More than 6000 hectares of suitable habitat were surveyed by field teams and fire ant odour detection dogs, and the size of the infested area was determined to be 220 hectares (Wylie et al. 2016).

In the three months following initial detection, samples from 66 colonies were analysed. A pedigree or family tree of the Port of Gladstone incursion was constructed

using a combination of approaches. R co-efficients were used to infer *S. invicta* relationships from Kingroup (Konovalov et al. 2004) and to manually add to and adjust the pedigree to best fit the data. Manual application allowed for flexibility and possibilities such as inbreeding to be accounted for. Bottleneck (Cornuet and Luikart 1996) analysis was conducted to determine heterozygosity excess or deficit. From these analyses it was concluded that:

a)   the incursion was likely founded by two queens;

b)   the population was in a distinct genetic bottleneck, with a strong bias towards heterozygosity excess associated with a very recent founding event;

c)   the incursion had been present for approximately six generations, which establishes its minimum age at around three years, about the time this section of the Port was constructed;

d)   fourteen colonies needed to complete the pedigree tree were not sampled, five of which are at the top of the tree and may not have survived. Therefore, it is estimated that only nine colonies were not identified through the surveillance activities, which equates to an efficacy rate of 88%. Four of those colonies were subsequently detected, which increases the efficacy rate of combined human and odour detection dog surveillance at the site to 93%;

e)   inbreeding/breeding with kin was notably high, particularly during the first generations;

f)   the pedigree tree depicts near-exponential amplification of the infestation;

g)   the mean post-mating flight distance for the mainland infestation was 420 m, ranging from 29 m to 1.2 km.

There were two long-distance movements to nearby Curtis Island of 3.8 km and 4.6 km, but it is not known if this was flight or via human assistance. The majority of flights (70%) were from the west to the east, which is against the prevailing onshore wind (from the east for 11 months of the year according to local meteorological data). This differs from the results of a United States study where Rhoades and Davis (1967) found 90% of newly founded colonies downwind from their source population. It is generally agreed that *S. invicta* favour highly disturbed habitats (see Tschinkel 2006) and this is also supported by unpublished program data from Brisbane where approximately 70% of all finds are associated with major soil disturbance, predominantly residential, commercial and road development. At the Port of Gladstone, the presence of a large expanse of suitable habitat at the site (specifically, bare ground) may have had a greater influence on the establishment of newly mated queens than wind direction (Fig. 3).

We found evidence that the Gladstone infestation experienced pleiometrosis (collaborative founding of nests by multiple monogyne queens) which can result in relatively higher fitness of colonies. Pleiometrosis has been reported previously in the United States (Tschinkel and Howard 1983; Bernasconi and Strassman 1999; Manfredini et al. 2013) and can result in higher survival and growth rates in the claustral period (when the first generation of workers is raised) than for nests founded by a
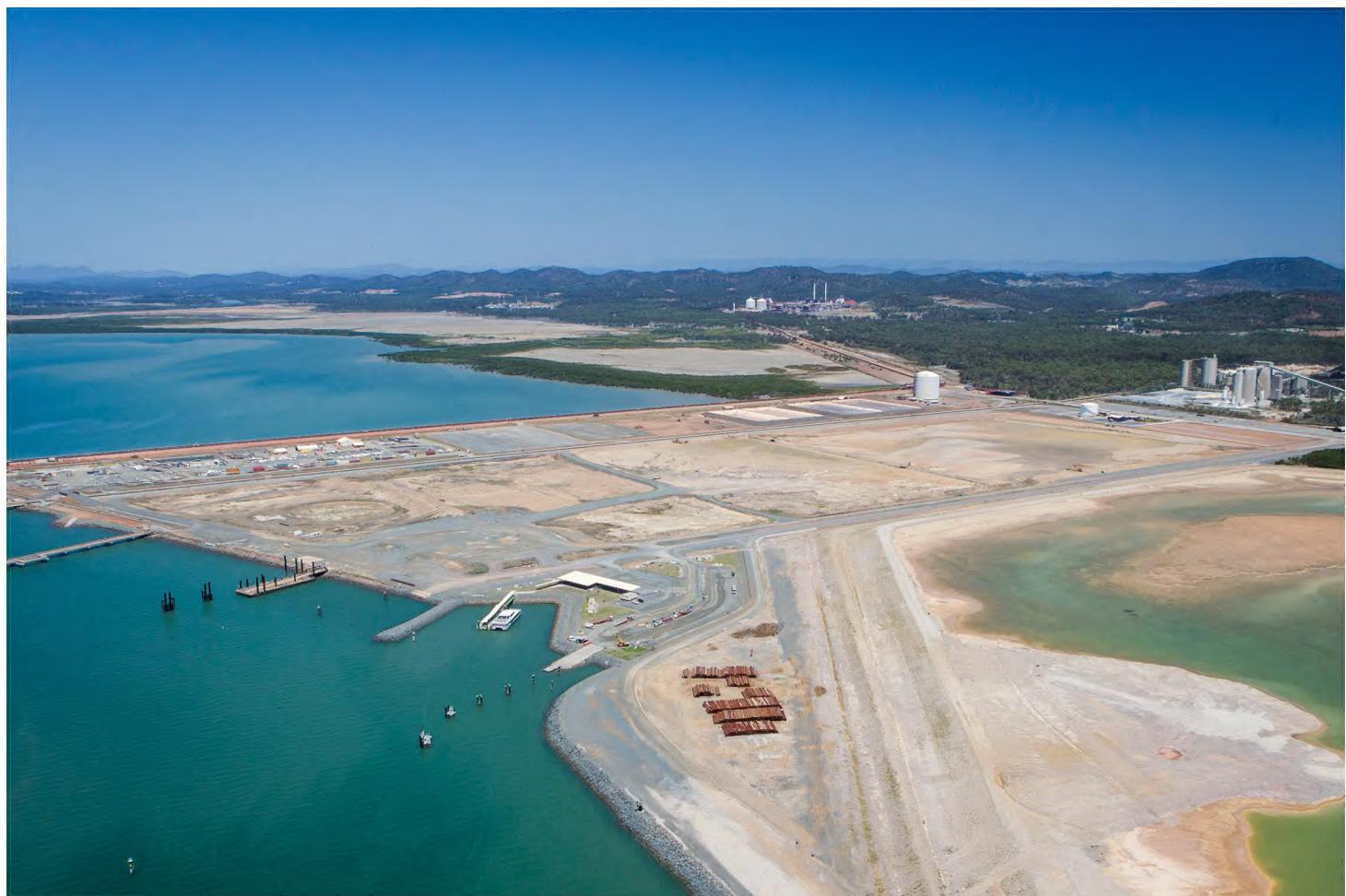
Figure 3. Photograph of Fisherman's Landing, Port of Gladstone in central Queensland. The large expanse of bare ground was attractive habitat for newly mated *S. invicta* queens, with the majority of nests found on this site along the edges of drains where moisture was present or in isolated grass clumps.

single queen. Ultimately, only one queen will survive and gain reproductive dominance. We concluded pleiometrosis was likely because, unexpectedly for monogyne colonies, over 20% of the nest samples were found to consist of workers from more than one queen and over half of these had 2–4 families present. In these cases, the dominant genotype was accepted as the nest identity, and the other workers labelled as 'tourists'. While one explanation for the 'tourists' is poor sampling technique resulting in mixed samples, this is unlikely given that the operations teams were all experienced and such a result from the same teams had not occurred previously. Another possibility is that samples contained foragers from surrounding nests as well as in-nest workers. Monogyne colonies are generally aggressive to workers from other colonies, although the high level of inbreeding and genetic inter-relatedness of nests may confuse the recognition of self/kin and territorialism that would normally combat or repel the 'tourists'.

Pleiometrosis is more common where mated queens are in high density or where suitable habitat is scarce. This is consistent with the main site of infestation at the Port of Gladstone, which was challenging for colony founding, being reclaimed sand and coral fragments and a 'hard stand' of compressed crusher dust and gravel. The majority of the nests found in those areas were along the edges of drains where moisture was present or in isolated grass clumps. This potential pleiometrosis at the Port of Gladstone may be related to 'microtopography' (limited suitable habitat available in the

landscape resulting in clumping of founding queens (Tschinkel and Howard 1983). As most 'tourists' could not be assigned to a source nest, it is postulated that they may be residual workers from pleiometrotic founding events.

Remote sensing surveillance

Background

The detection and delimitation of the extent of the infestation is one of the major challenges with invasive ant incursions. Indeed, it has been purported that an inability to detect all nests will either expand the time and cost of eradication, or inevitably lead to failure to eradicate (Hoffmann et al. 2010). In the early years of the program, surveillance was conducted solely by on-ground field staff, either visually or using lured pitfall traps or 'hot dog' sausage lures. When there was visible mound building or soil disturbance by the ants (usually in the cooler months or after heavy rain), visual surveillance provided detection rates of around 80% based on quality assurance evaluations. However, human visual surveillance is expensive and slow, as are pitfall traps and lures. Early in the program, as infestations began to be detected in less-populated peri-urban and rural areas, it became apparent that a more efficient method was needed for large-area surveys. A range of methods was investigated, with aerial remote sensing selected as the most suitable option for broad scale surveillance. Initial trials conducted in late October 2004 using infrared and colour video cameras to detect *S. invicta* mounds were unsuccessful. This was attributed to technical limitations for image capture, data storage and processing, and the timing of flights in warm weather when there was little temperature differential between the background and the target (Biosecurity Queensland, unpublished data). Subsequent advances in technology combined with successful trials of remote sensing for *S. invicta* in the United States (Vogt 2004a, b; Vogt et al. 2008a, b) led to the program's reinvestigation into the use of remote sensing in mid-2008.

Impetus to move remote sensing surveillance out of research and development and into operation came following an independent review of the program conducted in 2009–2010. The review concluded that eradication of the remaining *S. invicta* population in southeast Queensland was not feasible using existing techniques and recommended that research on remote sensing surveillance be completed within two years. Remote sensing surveillance became operational in 2012 with the primary aim of delimiting the southeast Queensland infestation.

Early remote sensing surveillance 2012–2015

Early research and development into remote sensing in the program realised three main technological components in the remote sensing surveillance process: capturing aerial imagery, analysing the imagery to identify potential *S. invicta* mounds,

and follow-up field surveillance to investigate those potential mounds. The camera system used by the program during this period was developed collaboratively by an Australian company (Outline Global Pty. Ltd.) in partnership with United States companies that had approved access to patented technology under the control of the US military. The system consisted of a camera pod containing six discrete high-resolution cameras; three visible spectrum (red, green, blue), one near-infrared and two long-wave thermal infrared. The cameras were chosen to maximise the chance of detecting *S. invicta* mounds through analysis of size, shape, colour, texture, vegetation cover and heat. The camera pod was mounted to the undercarriage of a helicopter that flew at a height of approximately 400 feet above ground level at a minimum speed of 30 knots.

Image capture

Remote sensing image capture was conducted in the cooler months of the year (May to September in Brisbane) when *S. invicta* mound temperatures can be considerably warmer than the surrounding ground. For example, in Brisbane, differences of up to 20–30 °C (average +11.9 °C, n = 1467) have been recorded, making them highly visible with thermal imagery, whereas recorded temperature differences were only up to 10 °C between mounds and their surroundings in Mississippi studies (Vogt et al. 2008a). As reported from remote sensing trials in the United States, a range of factors can affect the capture of suitable imagery, including nest location (obscured or in the open), mound shape, weather conditions, time of year and soil type (Vogt 2004a, b; Vogt et al. 2008a, b; Vogt et al. 2009). Similar findings were made in southeast Queensland and incorporated into operational guidelines. For example, one of the main factors affecting the thermal signature of a mound is cloud cover. Field observations in 2012 demonstrated that mounds cool rapidly when the sun is blocked by cloud, and within half an hour can be the same temperature as the surrounding ground. From this, an Aerial Operations Weather Guide and Ratings System was developed to help guide decisions on the suitability of conditions for flying. Flying is only conducted at Rating 1 (no visible clouds in the target area or the surrounding areas) or Rating 2 (high cloud and less than 10% of the sky with clouds). Mound temperature monitoring also provided information on the optimal times of day to fly; no earlier than 9 am and no later than 3.30 pm, except on the longer days in early spring. However, around the time of the winter solar azimuth when the sun is at its lowest angle, flights did not commence until 10 am and finished at 3 pm. It was also determined that mounds could be detected on the day following heavy rain provided that the mound was exposed to the sun. However, mounds in long grass were not detected unless the sun was overhead, and mounds at the bases of trees or in tree shade were difficult to detect. The shape of the mound was also important – dome-shaped mounds with a height of more than 15 cm provided the best thermal signature, while mounds that had been flattened and disturbed (e.g., by mowing) were generally undetectable.

Windy conditions may cause aircraft vibrations that resulted in image distortion, and steep terrain occasionally made it difficult for the pilot to maintain a consistent speed and height above the ground.

Image processing algorithm

Imagery was first georeferenced to establish the image location in respect to map projections and coordinate systems, and orthorectified to remove the effects of image perspective (tilt) and relief (terrain). The processed imagery was then analysed using a custom-designed machine-learning algorithm. One of the limitations in the development of the algorithm used in the 2012–2015 period was a lack of training images. At that time, focus was given to killing colonies as quickly as possible rather than retaining suitable sites for image capture. Although the algorithm had high detection rates when settings were adjusted to ensure no false negatives, it produced too many false positive points of interest for staff to follow up with field surveillance. Consequently, a manual analysis process was introduced whereby each point of interest identified by the algorithm was then assessed by a trained technician who would recommend to either discard or follow-up on a point. Using these two systems, and with further training of the algorithm, points of interest were reduced to operationally acceptable levels of about two per hectare.

Field surveillance

Point of interest surveillance involved field staff navigating to defined coordinates identified by the algorithm and manual analysis process, and then conducting a search in a 10 m radius around that point to confirm whether or not an *S. invicta* colony was present. If a mound was detected then an additional 500 m of field surveillance was conducted to detect any additional mounds in the area, this being the distance limit for 90% of *S. invicta* alates as determined by program genetics (see case study above) and work in the United States (Tschinkel 2006). Surveillance was extended if new mounds were found until there were no further detections.

Remote sensing results

Over the period 2012–2015, a total of 218 000 hectares of remote sensing surveillance was completed on the fringes of the known infested area to delimit the infestation at that time. When a new detection was confirmed, then the next round of surveillance was pushed out to 5 km beyond that detection, as this is the estimated distance limit for a newly mated queen to fly and successfully establish a colony (Vogt et al. 2000). Data obtained from remote sensing and from other program surveillance activities conducted during that period were analysed by a spread model developed by Keith et al. (2019) who concluded that the southeast Queensland infestation had been delimited with a 99.9% level of confidence as of May 2015.
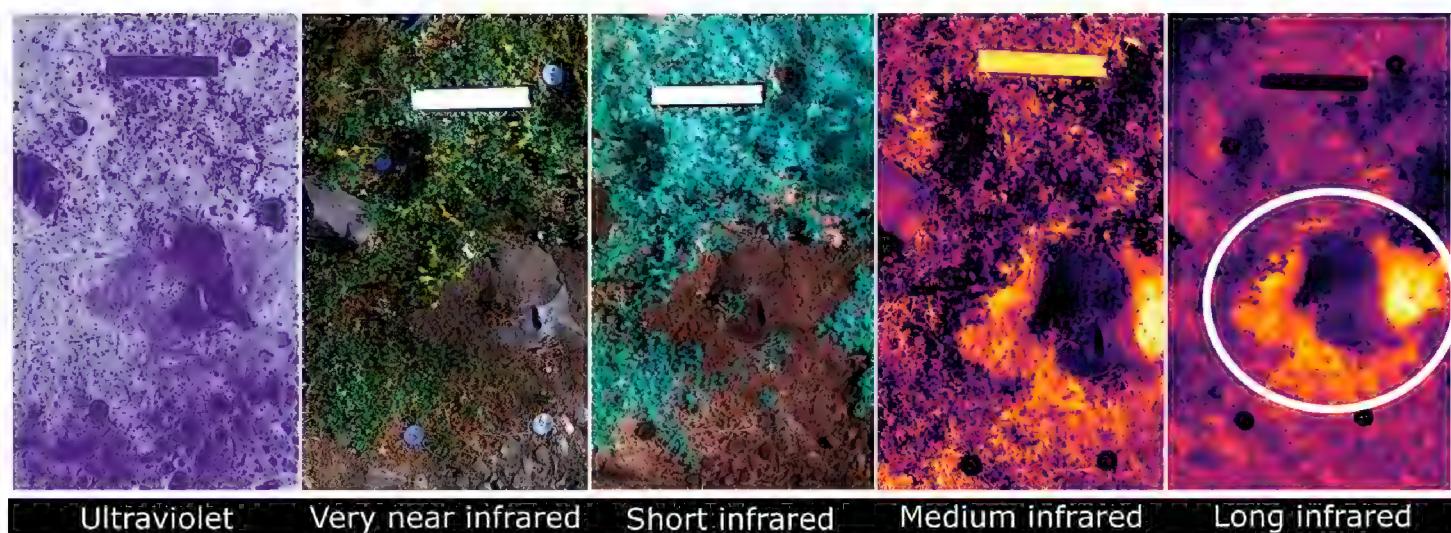
Figure 4. A comparison of the sensors investigated for use in detecting fire ants by remote sensing. Imagery was captured from a helicopter at 700 ft, with the five sensors including (from left to right): ultraviolet, very near infrared, short wavelength infrared, medium wavelength infrared and long wavelength infrared. A confirmed fire ant nest is present around a rock in the middle of images (white circle in right image) and is particularly apparent as bright yellow in both medium and long wavelength infrared. Imagery gathered in collaboration with Outline Global Pty Ltd, Australia.

Future remote sensing surveillance

The 2012–2015 delimitation of the infestation in southeast Queensland was a key factor that led an independent review of the program in 2015–2016 to conclude that eradication of the pest was still technically feasible and in the national interest. The review panel recommended the continuation of the eradication program and the development of a new response plan (Magee et al. 2016). Remote sensing surveillance ceased in June 2015 because the cameras were near or past the maximum shutter actuations recommended, and in need of refurbishment or replacement. Acquisition of next-generation remote sensing technology was necessarily delayed until funding was secured for the new response program that commenced in July 2017. Research and development on improved systems for image capture (including additional bandwidths; Fig. 4) and analysis (artificial intelligence deep-learning algorithm), data storage and field surveillance is nearing completion and the first operational flights are planned for 2021.

Discussion

As outlined by Shoemaker et al. (2006) there have been numerous studies on the genetic structure of ants at various scales to assess, for example, relatedness within and between social groups and the nature of queen-worker conflict, or to determine patterns of dispersal and gene flow and reveal relationships between native and introduced populations of invasive species. In Australia's eradication program for *S. invicta*, genetics is used as an everyday operational tool to guide the immediate eradication effort.

For example, knowledge of the social form of a new detection determines the extent of the search area around that detection, what treatment will be applied to the site and, if polygyne, whether investigations into human-assisted movement are warranted. Similarly, circumstances of a colony discovery may raise questions of non-compliance of movement restrictions of material containing monogyne alates, and the microsatellite genotype database is applied in an attempt to identify possible sources for investigation. Genetics is also used strategically to guide longer term planning and activities. Evidence of inbreeding and fragmentation in Brisbane *S. invicta* populations, implying reduced genetic fitness as a result of program activities, validates the program's efforts and bolsters the confidence of stakeholders that eradication is still achievable (see Magee et al. 2016).

Remote sensing surveillance similarly exploits aspects of the biology of *S. invicta*. The heat signatures of *S. invicta* mounds in the cooler months of the year can be captured in thermal imagery and their habit of keeping their mounds clear of vegetation is an additional marker that can be discerned by near infrared imagery as it produces a 'halo' effect around the mound. With the assistance of artificial intelligence algorithms, *S. invicta* mounds can be distinguished from those produced by other ant species such as meat ants (*Iridomyrmex purpureus*) or by mound-building termites in southeast Queensland (mainly grass-eating termites *Nasutitermes* species). The latest sensors and algorithm can also confidently distinguish *S. invicta* mounds from rocks and manufactured objects. Remote sensing surveillance has a key role to play in the ongoing program both for delineating the extent of the infestation and also assisting in verifying eradication of *S. invicta* in treatment areas as part of the proof of freedom process.

Whilst remote sensing has been used in pest management to detect changes in the environment caused by insects and fungal pathogens e.g., changes in plant health based on canopy spectral signatures (Stone and Mohammed 2017; Prasannakumar et al. 2020) and has been used widely to detect invasive plants (Bradley 2014; Niphadkar and Nagendra 2016), its use in invasive ant establishment is in its infancy. Considering the potential capability of remote sensing to detect fire ant nests over large areas (tens of thousands of hectares), this would provide a more efficient method compared to current ground surveillance activities.

Conclusion

Our program is the first time genetics and remote sensing surveillance have been used as routine, operational tools in an ant eradication program and particularly at the scale employed in southeast Queensland. Unquestionably, without these tools, the conclusions of the 2015–2016 independent review would have been very different, and the focus of the program would then have switched from eradication to managing and slowing the spread of the pest as has been necessary in other countries. The approaches we have used increase the chances of eradication of the red imported fire ant, thus

avoiding the considerable economic and environmental impacts of this invader, which would be to Australia's detriment. These methods and frameworks could be applied to management and eradication efforts for *S. invicta* worldwide. Potentially, these technologies could be adapted for use against other invasive ant species.

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